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The Human FK506-Binding Proteins: Characterisation of Human FKBP19

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Abbreviations: FKBP: FK506-binding protein; EST: expressed sequence tag

Abstract

Analysis of the human repertoire of the FK506-binding protein family of peptidyl-prolyl *cis/trans* isomerases (FKBPs) has identified an expansion of genes that code for human FKBPs in the secretory pathway. There are distinct differences in tissue distribution and expression levels of each variant. We describe here the characterisation of human FKBP19 (Entrez Gene ID: FKBP11), an FK506-binding protein predominantly expressed in vertebrate secretory tissues. The FKBP19 sequence comprises a cleavable N-terminal signal sequence followed by a putative peptidyl-prolyl *cis/trans* isomerase domain with homology to FKBP12. This domain binds FK506 weakly *in vitro*. FKBP19 mRNA is abundant in human pancreas and other secretory tissues and high levels of FKBP19 protein are detected in the acinar cells of mouse pancreas.

Keywords

FK506, petidyl-prolyl *cis/trans* isomerase, PPIase, pancreas

Introduction

The synthesis, folding and assembly of proteins involves some of the most essential and conserved mechanisms of life (Pahl et al., 1997). In eukaryotic systems, several families of chaperones and foldases have evolved to catalyse these processes (Fedorov and Baldwin, 1997). The folding of proline-containing polypeptides is catalysed by peptidyl-prolyl *cis/trans* isomerases (PPIases: Fischer et al., 1984).

The PPIase families are classified by sequence homology and pharmacologically by their ability to bind the immunosuppressant compounds cyclosporin, FK506 and rapamycin, and are otherwise known as immunophilins (reviewed in Galat and Riviere, 1998, Galat, 2003). The FK506-binding protein (FKBP) family share a high degree of sequence and structural homology and PPIase activity that is specifically inhibited by FK506 or rapamycin (Kay, 1996). Since the discovery of the first FKBP (Siekierka et al., 1989), several members of this family have been characterised in humans and other organisms (Patterson et al., 2002, Galat, 2003). Each member of the FKBP family contains one or more PPIase domain, which shows high sequence homology with the most abundant member, FKBP12.

Analysis of the genomes of eukaryotic model organisms shows correlations between genome size and the number of genes coding for FKBPs, with 4 members present in *Saccharomyces cerevisiae*, 7 in *Drosophila melanogaster*, and 9 in *Caenorhabditis elegans* (Kay, 2000). In general, eukaryotic organisms appear to have one or two FKBPs for every 2,000 genes in their genome. If this pattern applied to the human genome, containing around 30,000 genes (Pennisi, 2003), there could be up to 30

FKBP sequences in the human genome. Although many cellular processes involving FKBPs may be conserved from lower organisms, and degeneracy involving cyclophilins or parvulins as alternative PPIases may significantly reduce this predicted number. This report describes a survey of the human genome for FK506-binding proteins and the biochemical characterisation of a novel member, FKBP19.

Methods

Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich Company Ltd, Poole, UK.

Cloning

IMAGE clones containing FKBP sequences were obtained from Research Genetics Inc. (Huntsville, AL, USA). These included IMAGE clone 269896 (accession numbers N24885 and N36303), containing the full-length FKBP19 coding sequence, clone 323084 (accession number W42674), containing a partial FKBP22 sequence, and clones 2518850 and 1857997 (accession numbers AI879695 and AI271550), which contained overlapping sequences covering the entire FKBP23 coding sequence. The FKBP23 clones shared a common internal StuI site. A BamHI-StuI fragment of the IMAGE clone 2518850 insert, and a StuI-NotI fragment of clone 1857997 were generated. A three-way ligation of these two fragments with a BamHI-NotI digested pET21a expression vector produced a full-length human FKBP23 clone.

Northern Blotting

Probes were generated to the open reading frames of each FKBP variant shown by PCR and radiolabelled with α-^{32}P dCTP using a Megaprime labelling kit (Amersham Pharmacia Biotech Ltd. Little Chalfont, UK). These were then used to probe a Human

Multiple Tissue Northern and Human RNA Master Blot (Clontech) using ExpressHyb (Clontech), according to the recommended protocols. Washed blots were exposed to a phosphorimager screen (Molecular Dynamics, Sunnyvale, CA).

In vitro transcription/translation of hFKBP19

A full-length FKBP19 expression construct was generated by PCR using primers FKBP19/3 and FKBP19/4 (see figure 3) and the resulting product subcloned into pET21a using restriction sites incorporated into the primer sequences. The construct was then used as a template in the TNT-T7 Quick Coupled Transcription/Translation System (Promega UK Ltd, Southampton, UK). Translation products were radiolabelled with ^{35}S-methionine (Amersham Pharmacia Biotech Ltd. Little Chalfont, UK). Post-translational peptide cleavage was obtained by carrying out the coupled reaction in the presence of Canine Pancreatic Microsomal Membranes (Promega).

Expression constructs

Full-length expression constructs of FKBP19 and FKBP23 produced insoluble material in *E.coli*, so PCR primers (Genosys Biotechnologies Ltd, Cambridge, UK) were designed to amplify regions coding for the PPIase domain of human FKBP19 (GAGGCTGGGCTCGAAACCG andGCCCTTCACCAGCTTTAGCC) and the "mature" FKBP23 (complete ORF minus the first 24 amino acids, using primers 5'-CCCATATGAGACAAAAGAAAGAGGAGAGC-3' and 5'-CCCTCGAGTAGTTCATCGTGTTGGTATAC-3'). An in-frame start codon was incorporated, where necessary, into PCR primers, as part of an NdeI site, and an XhoI site was incorporated at the 3' end, replacing the native stop codon. The resulting PCR fragment was cloned into pET21a (Novagen, Madison, WI, USA), such that the FKBP coding sequences were in-frame with a C-terminal His-tag sequence. A similar His-

Tagged expression construct was generated from the full-length coding region of human USA-CYP for use as a negative control for binding studies as previously described (Pemberton et al., 2003). An FKBP13 expression construct was made for use as a positive control by amplifying a human FKBP13 fragment coding for the "mature", soluble form of the protein (i.e. the full-length minus the first 21 amino acids) with primers 5'-CCGAATTCACGGGGGCCGAGGGCAAAAGG-3' and 5'-CCAAGCTTCAGCTCAGTTCGTCGCTCTAT-3'. The fragment was subcloned into pET21a using the EcoRI and HindIII sites incorporated into the primer sequences.

Expression and purification of human PPIases

BL21(DE3) cells (Novagen) transformed with each expression construct were grown in flasks, shaking at 200 rpm, at 37°C in 2YT broth supplemented with 50 µg/ml ampicillin, 1% glucose. Expression was induced at an OD_{600} of 0.8 with 1mM IPTG. Cells were harvested 2.5 hours after induction. Inclusion body preps were made from FKBP19-expressing *E.coli* as follows: 1g of cells were lysed by sonication in 10 ml 50 mM sodium phosphate (pH 7.0), 300 mM NaCl, 0.5 mM PMSF, and centrifuged at 1000g, 15 minutes, to pellet inclusion bodies. These were washed twice in lysis buffer and resuspended in lysis buffer containing 6 M guanidine-HCl. After 4 hours mixing at 4º, the samples were centrifuged again, and the supernatant was added to 1 ml TALON resin (Clontech, Basingstoke, UK). After mixing at 4ºC for 1 hour, the resin was collected by centrifugation, (1000 g, 5 minutes), washed twice in 10 ml lysis buffer containing 6 M guanidine-HCl and 3 times in guanidine-free lysis buffer. Purified FKBP19 was eluted in 1ml fractions with lysis buffer containing 150 mM Imidazole.

His-tagged FKBP23 was purified as described above, with the exception that the protein was purified from the soluble fraction of the cell lysate, and no guanidine-HCl was used at any stage. FKBP13 was purified on SP Sepharose resin in 50 mM Bis-Tris Propane buffer at pH 9 with a 0-1 M NaCl gradient. Fractions giving the correct size band were dialysed into 50 mM Tris, 200 mM NaCl, pH 7.4. Human USA-CYP was purified as described (Pemberton et al., 2003).

Antibodies and Western Blotting

Protein products and *in vitro* translation products were separated by SDS-PAGE using precast 4-20% Tris-Glycine gels (Invitrogen) and transferred to PVDF membrane (Invitrogen). Blots were blocked in 5% dried milk for 1hr, incubated with primary antibody/1% milk in PBS for 2hrs, washed in PBS/0.1% Tween-20 three times, incubated in secondary antibody/1% milk for 1hr and washed 5 times in PBS/0.1% Tween-20. Blots were developed using BCIP/NBT liquid substrate system (Sigma).

For analysis of tagged protein expression in *in vitro* translation products and *E.coli* extracts, an alkaline phosphatase-conjugated monoclonal anti-T7 antibody was used (Novagen) and incubation with secondary antibody was not necessary. For analysis of native protein expression in bovine and mouse extracts, a rabbit polyclonal antibody was generated to the full-length tagged protein product by Cambridge Research Biochemicals. The specificity of this antibody was further improved by purifying PPIase-domain-binding species in the immunised rabbit serum using a Hi-Trap NHS-activated column (Amersham) containing conjugated FKBP19 PPIase domain. An alkaline phosphatase-conjugated anti-rabbit IgG antibody was used as a secondary antibody.

^{3}H-FK506-Binding Assay

The FK506-binding assay (Siekierka et al., 1990) was carried out in individual 1ml columns prepacked with LH20 medium (Amersham Pharmacia Biotech Ltd. Little Chalfont, UK) and equilibrated in assay buffer (50 mM Tris-HCl, pH 7.4, 200 mM NaCl). 1 μM FKBP was incubated with 12 nM ^{3}H-dihydro-FK506 (NEN Life Science Products, Hounslow, UK) for 25 minutes at room temperature before being applied to the column. 100 μl fractions were eluted in assay buffer and counted for 10 minutes in a Beckman LS 6000IC scintillation counter.

Immunohistochemistry

Adult C3H mouse pancreas was fixed in 10% buffered formaldehyde and embedded in paraffin wax. 5um thick sections were obtained and de-paraffinised in xylene substitute and graded ethanol solutions. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide in isopropanol.

For FKBP19 labelling the sections were microwaved in 10mM citrate buffer (pH 6.0) and rinsed with phosphate-buffered saline at room temperature. To inhibit the non-specific endogenous biotin staining the DAKO Blocking System was used. A blocking solution of 10% swine serum (DAKOCytomation) was used for 10 minutes. Polyclonal anti-FKBP19 was used at a dilution of 1:100 for one hour at room temperature. Biotinylated swine anti rabbit antibody (DAKOCytomation) and ChemMate Detection Kit (DAKOCytomation) were used to develop the specific signals. The negative control specimen was incubated in swine serum and processed identically.

For insulin labelling the sections were rinsed with phosphate-buffered saline, blocked with 10% rabbit serum and incubated with polyclonal guinea pig anti-insulin

(DAKOCytomation) at a dilution of 1:500 for one hour at room temperature. Peroxidase-conjugated rabbit anti guinea pig (DAKOCytomation) and diaminobenzidine (DAKOCytomation) were used to develop the signals. The negative control specimen was incubated in rabbit serum and processed identically. All slides were counterstained with haemotoxylin.

Results

Fifteen FKBP sequences have been identified in the human genome (table 1). Only genes where EST-derived sequences are available are included here. Reference to hFKBP-12c (described by Galat, 2003) is omitted from these data since no expressed sequences corresponding to this predicted sequence were found. The FKBP family can be subdivided into four groups by classification of the regions within or surrounding the conserved PPIase domains (figure 1). In humans there are multiple subtypes within each of these groups. Each subtype may bind different subcellular targets as a result of subtle differences in their sequences. The four mammalian FKBP subgroups comprise the cytoplasmic, nuclear, TPR-domain, and secretory pathway FKBPs. The cytoplasmic FKBPs contain single FKBP domains only (FKBP12 and FKBP12.6: (Standaert et al., 1990, Arakawa et al., 1994)). The nuclear FKBPs (FKBP25 and FKBP135; (Jin et al., 1992, Ishikawa et al., 1998) contain N-terminal domains of unknown function, and one or more nuclear localisation sequences. The TPR-domain FKBPs contain two or three FKBP domains and tetratricopeptide repeats (TPRs) within the C-terminal FKBP domain (FKBP36, FKBP37, FKBP38, FKBP51 and FKBP52; (Meng et al., 1998, Kuzhandaivelu et al., 1996, Lam et al., 1995, Nair et al., 1997, Peattie et al., 1992).

The largest group of human FKBPs are the secretory pathway class of FKBPs (Davis, 2000), which comprise FKBP13 (Jin et al., 1991), FKBP19 (this article), FKBP22 (Patterson et al., 2002), two splice variants of FKBP23 (one of which was previously identified in mouse: Nakamura et al., 1998), FKBP60 (Shadidy et al., 1999), and FKBP65 (Patterson et al., 2002). Members of this group are distinguishable from other FKBPs by the presence of N-terminal, leucine-rich, cleavable signal peptides and C-terminal endoplasmic reticulum (ER)-retention motifs, which in most cases is a variant of the canonical KDEL motif (Munro and Pelham, 1987). Calcium-binding EF-hand motifs are also present in the C-terminal region of several of these proteins. The PPIase domains of this class of proteins can be identified by the presence of two cysteine residues, which are thought to form a disulphide bond in the oxidising environment of the ER and stabilise the tertiary structure of the PPIase domain (Jin et al., 1991). Other typical features of this group are the presence of two basic amino acids 4 residues downstream of the second cysteine residue and a conserved proline 5 residues thereafter (see figure 2).

FKBP19 (accession number AF238079) represents a previously uncharacterised FKBP subtype. The 740bp human FKBP19 cDNA sequence is derived from 6 exons on chromosome 12, and contains an open reading frame coding for a 22 kDa protein with some unique features. Highly conserved FKBP19 orthologues are present in other vertebrates (figure 3) but none have been found in invertebrate species.

A 4kb region of chromosome 12 that lies immediately upstream of FKBP19 was found in the Ensembl database and scanned for putative transcription factor binding sites using the Biobase Match^{TM} tool (Goessling et al., 2001). Five putative NKX2-5

and four Pax4 binding sites were found in this region, suggesting a role in the pancreas (Inoue et al., 1998). Analysis of EST sequences showed that FKBP19 is highly expressed in melanocytes and placenta, but is also expressed in pancreatic islet cell, senescent fibroblast and breast libraries. A radiolabelled FKBP19 probe showed a transcript of ~800bp expressed at high levels in human pancreatic tissue, with low expression levels in the other seven tissues probed, and very low levels in brain (figure 4, left panel). High levels of expression in the pancreas and other secretory tissues such as stomach, pituitary gland, salivary gland and lymph node, were confirmed by probing a dotblot containing messenger RNA extracted from 50 different human tissues (figure 4, right panel). In contrast, FKBP22 and the two FKBP23 variants, show low levels of expression in the secretory tissues, being expressed at relatively higher levels in the heart. However, differences between the expression levels of FKBP22 and the FKBP23s in the brain, for example, show that these members of the FKBP family also exhibit tissue-specific expression.

The FKBP domain of FKBP19 sequence demonstrates 38% amino acid identity with FKBP12 and 46% with the FK506-binding domain of FKBP13. It contains some of the features typical of secretory pathway FKBPs, such as the two cysteine residues, and also a conserved basic motif and subsequent proline residue shortly after the second cysteine (see figure 2). The FKBP domain of FKBP19 binds FK506 in an *in vitro* binding assay shows binding of FK506 over non-specific binding by a structurally-unrelated PPIase USA-CYP (Horowitz et al., 1997). However, FKBP13 binds over 200 times as much FK506 in the same assay, whereas the binding of FK506 by FKBP23 is not detectable over non-specific binding (figure 5).

Other features of FKBP19 include a leucine-rich N-terminal leader sequence of 25 residues, which shows similarities with other known secretory pathway proteins (von Heijne, 1983). Cleavage at the predicted site of 3 kDa would leave a 19 kDa mature protein, thus named FKBP19. The C-terminus of this protein differs from the other secretory-pathway FKBPs in that it lacks a calcium-binding EF hand that is typical of several other secretory pathway FKBPs and contains a putative transmembrane domain (as predicted by PSORT; Nakai and Horton, 1999), followed by a lysine-rich C-terminal tail, containing a variant of the di-lysine motif found in ER-membrane proteins (Teasdale and Jackson, 1996).

An antibody raised to the PPIase domain of FKBP19 recognised recombinant FKBP19, but not the closely related PPIases FKBP12.6 and FKBP23 (figure 6A). Affinity chromatography to an FKBP19-conjugated column completely abolished detectable antibody binding, showing that the antiserum is specific to FKBP19 (figure 6B). Anti-FKBP19 detects a doublet of 19-22 kDa in bovine pancreas extracts (fig 6D). This is likely to be a mixed population of proteins containing cleaved and un-cleaved N-termini. This is supported by the loss of approximately 3kDa in an *in vitro* reaction in the presence of canine pancreatic microsomes (figure 6E). Immunohistochemical analysis of FKBP19 production in the mouse pancreas shows high levels of FKBP19 protein, localised throughout the cytoplasmic region of acinar cells and concentrated in the perinuclear region of these cells (figures 7A and 7C). Low levels are seen in the islets of Langerhans, which were confirmed by immunolabelling with an antibody directed to insulin (figure 7D).

Discussion

Analysis of human genome sequence data has confirmed previous reports that there are fifteen different FKBP variants expressed in human tissues. It seems likely that this number represents the complete human FKBP repertoire, although splice variation in those such as FKBP23 may increase the number of different functional FKBPs. The conserved sequences of the PPIases suggest that their cellular targets may overlap. Common partners of TPR-containing PPIases have been demonstrated previously (Ratajczak and Carrello, 1996). Tissue-specific expression profiles, such as that shown for FKBP19 above, and the separation of multiple subtypes by subcellular localisation signals (figure 1) may be sufficient to avoid much of the erroneous binding by promiscuous members of this family. However, the overlapping expression profiles and subcellular localisation of FKBPs in the secretory pathway suggest that regulation of expression and protein sorting is not enough. This raises the question of why multiple isoforms are so highly conserved between species, if they are involved in degenerate pathways. Alternatively, subtle differences in the primary sequence of a protein could lead to dramatic differences in its affinity for a target. This hypothesis is supported by the differential binding abilities of three similar secretory-pathway FKBPs for the exogenous ligand, FK506. However, a third explanation could be that sequence divergence is *required* for tertiary structure and functionality in a wide range of cellular environments. The presence of a disulphide bond in the FKBP domain appears to be necessary for all of the human FKBPs in the secretory pathway. Extremes in pH, oxidative or ionic strength in tissues such as the pancreas may be why specialised molecules are required for functional protein production and transport in these tissues. pH-dependent activity has been demonstrated for other PPIases (Ranganathan et al., 1997).

There appears to be a wider expansion of secretory FKBPs in vertebrates compared to other animals. Analysis of genomic models shows that *S.cerevisae* has only one FKBP in the secretory pathway, whereas *Schizosaccharomyces pombe* has none at all. There are two present in the *Drosophila* genome, and up to 5 in *C.elegans* (Kay, 2000). This report confirms that at least six secretory pathway FKBPs are present in vertebrates: FKBP13, FKBP22, FKBP23 (isoforms a and b), FKBP60 and FKBP65 represent closely-related variants of the FKBPs in the ER lumen, although FKBP13 lacks the calcium-binding region of the other members of this group. These probably have similar functions in folding proteins in the bulk flow of the ER of these tissues, as previously suggested for FKBP13 (Bush et al., 1994). FKBP19 represents a highly conserved unique FK506-binding protein, which so far appears to be unique to vertebrate genomes. Unlike FKBP65, FKBP19 does not appear to be developmentally regulated, and is expressed at low levels in many fetal and adult tissues. FKBP19 may be bound to the membrane of secretory microsomes and expression appears to be elevated in tissues with exocrine properties. Highly tissue-specific PPIases have been previously reported: For example the cyclophilin protein ninaA is specifically expressed in the photoreceptor cells of *Drosophila* and is required for the production of rhodopsin (Baker et al., 1994). The expression profile of FKBP19 suggests that its role may have a unique role in protein secretion. Identification of cellular targets would be required to confirm this hypothesis.

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Table legends

Table 1:

Identification of human EST sequences encoding FKBP-domain proteins. FKBPs are grouped by subfamily (see results section: C = cytoplasmic, N = nuclear, TPR = TPR-containing, ER = secretory pathway). Locuslink Ids and chromosomal positions as recorded in the NCBI Locuslink database are shown.

Figure legends

Figure 1:

Domain structure of the 13 human FKBPs.

Figure 2:

Clustal alignment of the PPIase domains of the known human FKBPs. FKBPs are grouped by class (C = cytoplasmic, N = nuclear, TPR = FKBPs with tetratricopeptide repeats, ER = secretory-pathway). Multiple PPIase domains within the same sequence are shown in order from the N-terminal domain. Residues interacting with FK506 are shown in bold (from the FKBP12 model: Ikeda et al., 1994)). Regions forming the alpha helix (α) or beta sheets (β) within the PPIase domain are also shown (Michnick et al., 1991). The two cysteine residues found in secretory-pathway FKBPs are indicated with arrows, and the nuclear localisation motif of FKBP25 is underlined.

Figure 3:

Clustal alignment showing the predicted amino acid sequences derived from putative FKBP19 homologue EST sequences (Thompson et al., 1994). Accession numbers as follows: *Bos taurus* (derived from two overlapping ESTs: AV589801 and AV615018); *Rattus norvegicus*: BF555031 and AA875098; *Mus musculus*: NM_024169, *Xenopus laevis*: BC078481; *Dani rerio*: AL591389. "*" = identical or conserved residues in all sequences in the alignment ":" = indicates conserved substitutions "." = indicates semi-conserved substitutions. Amino acids predicted to comprise the PPIase domains are in bold. The cysteine residues specific to secretory-pathway FKBPs are underlined. The predicted N-terminal signal sequences and transmembrane regions have dotted underlines.

Figure 4:

(Left) Clontech's Human Multiple Tissue Northern was probed with a ^{32}P-labelled probe generated from the coding sequences of FKBP19, FKBP22 or FKBP23 Approximate sizes of transcripts are shown on the left. Lanes represent mRNA isolated from (1) heart, (2) brain, (3) placenta, (4) lung, (5) liver, (6) skeletal muscle, (7) kidney, (8) pancreas. (Right) Results obtained by using the FKBP19-specific probe with a Clontech human mRNA Master Blot, which contains normalised RNA samples from 42 different human tissues: A1-8: whole brain, amygdala, caudate nucleus, cerebellum, cerebral cortex, frontal lobe, hippocampus, medulla oblongata; B1-7: ocipital lobe, putamen, substantia nigra, temporal lobe, thalamus, sub-thalamic nucleus, spinal cord; C1-8: heart, aorta, skeletal muscle, colon, bladder, uterus, prostate, stomach; D1-8: testis, ovary, pancreas, pituitary gland, adrenal gland, thyroid gland, salivary gland, mammary gland; E1-8: kidney, liver, small intestine, spleen ,

thymus, peripheral leukocyte, lymph node, bone marrow; F1-4: appendix, lung, trachea, placenta; G1-7: fetal brain, fetal heart, fetal kidney, fetal liver, fetal spleen, fetal thymus, fetal lung; H7-8: human gDNA 100 ng, human gDNA 500 ng.

Figure 5:

FK506-binding by FKBP13 (left panel), FKBP19 or FKBP23 (right panel). 1 μM protein was preincubated with 12 nM ^{3}H-dihydro-FK506 before bound compound was separated from unbound on a 1.8ml LH20 column. 100μl fractions were assayed by liquid scintillation.

Figure 6:

Western blot showing the specificity of anti-FKBP19 antiserum. Each lane contains equivalent loadings (0.4μg) of protein from the following: (1) lysate from IPTG-induced *E.coli* containing an expression construct for His-tagged FKBP19 PPIase domain (see methods); (2) purified recombinant FKBP12.6 (3) semi-purified recombinant FKBP23. Panel A shows a blot probed with anti-FKBP19 after elution from a solid-phase "blank" affinity column; (B) Western blot probed with serum eluted from an FKBP19-conjugated column (see methods); (C) Coomassie stained gel of the same samples. (D) Bovine pancreas extract probed with anti-FKBP19. (E) Western blot of an *in vitro* FKBP19 translation product carried out in the absence (-) or presence (+) of canine pancreatic microsomes (see methods).

Figure 7:

Immunohistochemical localisation of FKBP19 in mouse pancreas sections. (A) Pancreas section incubated with affinity-purified anti-FKBP19 (20x) showing

labelling of the exocrine tissue; (B) negative control showing staining produced with secondary antibody incubation; (C) 60x view of FKBP19 immunostaining; (D) adjacent section to that shown in (C) immunolabelled for insulin to show positive staining in the beta cells of the islets of Langerhans, and negative staining in the exocrine region of the pancreas.

Table 1:

FKBP	Refseq ID	Locuslink	Class	Chromosome
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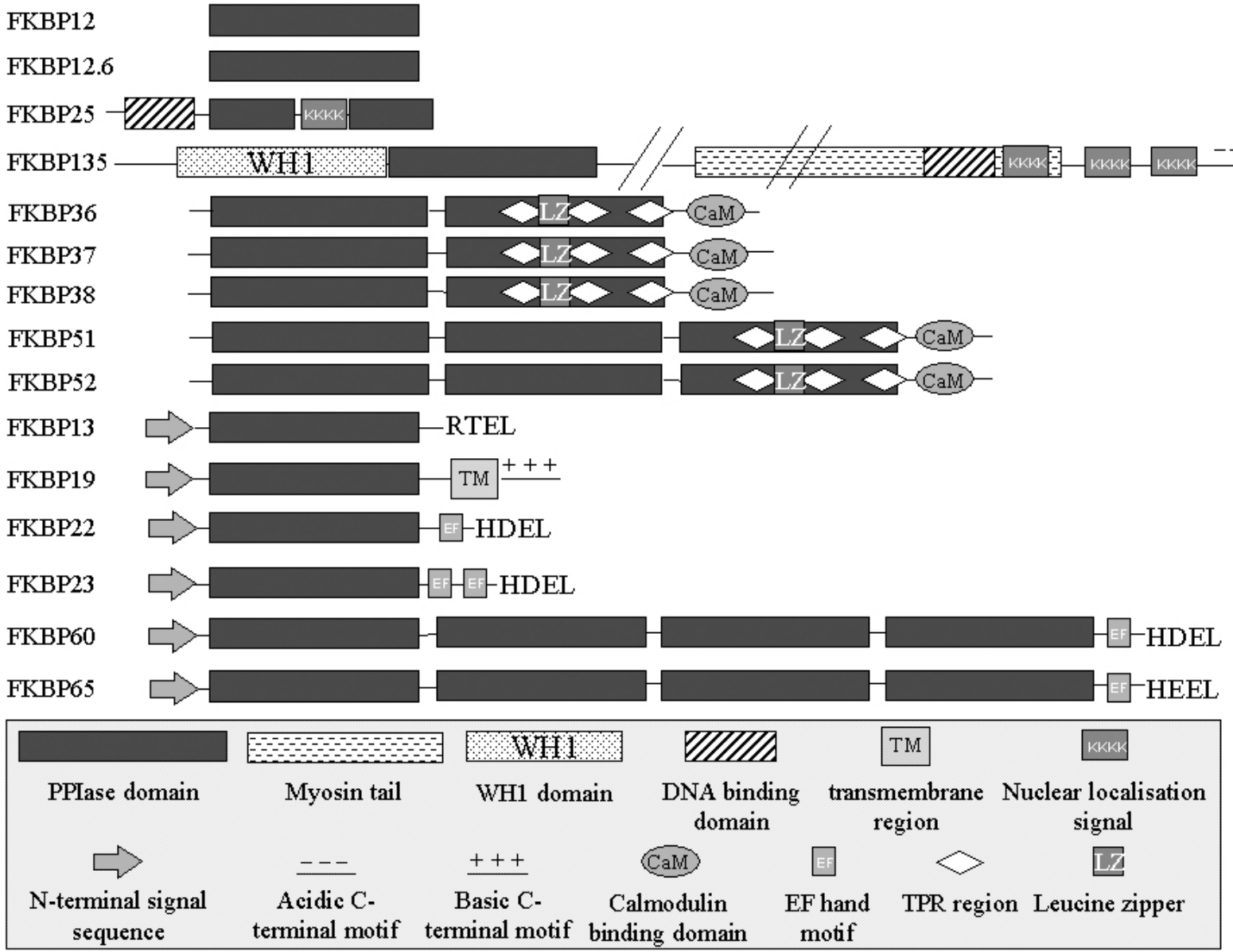
FKBP135	KIAA0674	23307	N	9q32
FKBP36	FKBP6	8468	TPR	7q11
FKBP37	AIP	9049	TPR	11q13
FKBP38	FKBP8	23770	TPR	19p12
FKBP51	FKBP5	2289	TPR	6p21
FKBP52	FKBP4	2288	TPR	12p13
FKBP13	FKBP2	2286	ER	11q13
FKBP19	FKBP11	51303	ER	12q13
FKBP22	FKBP14	55033	ER	7p15
FKBP23	FKBP7	51661	ER	2q31
FKBP60	FKBP9	11328	ER	7p11
FKBP65	FKBP10	60681	ER	17q21

Figure 1: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FKBP135
WH1
KKKK
KKKK
KKKK
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CaM
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LZ
CaM
FKBP51
LZ
CaM
FKBP52
LZ
CaM
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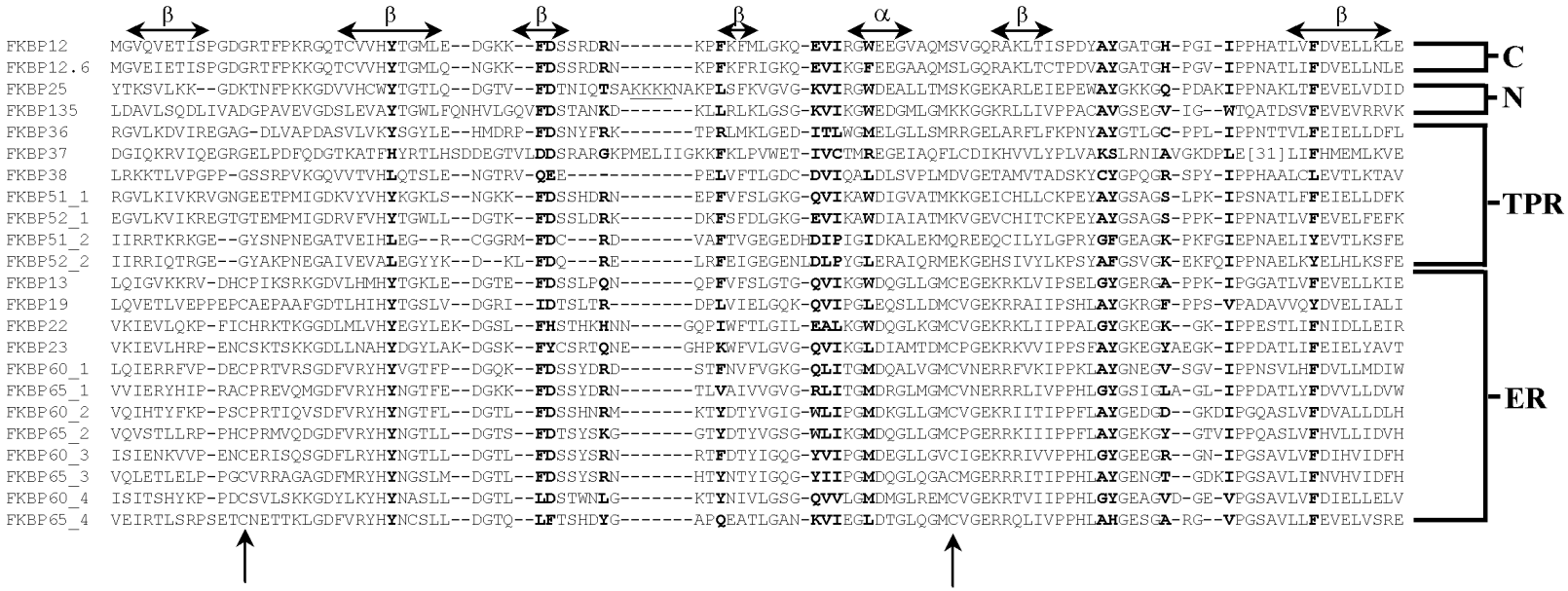
RTEL
FKBP19
TM
+ + +
FKBP22
EF
HDEL
FKBP23
EF
EF
HDEL
FKBP60
EF
HDEL
FKBP65
EF
HEEL
PPIase domain
Myosin tail
WH1
WH1 domain
DNA binding domain
TM
transmembrane region
KKKK
Nuclear localisation signal
N-terminal signal sequence
– – –
Acidic C-terminal motif
+ + +
Basic C-terminal motif
CaM
Calmodulin binding domain
EF
EF hand motif
TPR region
LZ
Leucine zipper

Figure 2: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FKBP12.6 MGVEIETISPGDGRTFPKKGQTCVVHYTGMLQ--NGKK--FDSSRDRN-------KPFKFRIGKQ-EVIKGFEEGAAQMSLGQRAKLTCTPDVAYGATGH-PGV-IPPNATLIFDVELLNLE
FKBP25 YTKSVLKK--GDKTNFPKKGDVVHCWYTGTLQ--DGTV--FDTNIQTSAKKKKNAKPLSFKVGVG-KVIRGWDEALLTMSKGEKARLEIEPEWAYGKKGQ-PDAKIPPNAKLTFEVELVDID
FKBP135 LDAVLSQDLIVADGPAVEVGDSLEVAYTGWLFQNHVLGQVFDSTANKD-------KLLRLKLGSG-KVIKGWEDGMLGMKKGGKRLLIVPPACAVGSEGV-IG--WTQATDSVFEVEVRRVK
FKBP36 RGVLKDVIREGAG-DLVAPDASVLVKYSGYLE--HMDRP-FDSNYFRK-------TPRLMKLGED-ITLWGMELGLLSMRRGELARFLFKPNYAYGTLGC-PPL-IPPNTTVLFEIELLDFL
FKBP37 DGIQKRVIQEGRGELPDFQDGTKATFHYRTLHSDDEGTVLDDSRARGKPMELIIGKKFKLPVWET-IVCTMREGEIAQFLCDIKHVVLYPLVAKSLRNIAVGKDPLE[31]LIFHMEMLKVE
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FKBP52_1 EGVLKVIKREGTGTEMPMIGDRVFVHYTGWLL--DGTK--FDSSLDRK-------DKFSFDLGKG-EVIKAWDIAIATMKVGEVCHITCKPEYAYGSAGS-PPK-IPPNATLVFEVELFEFK
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FKBP13 LQIGVKKRV-DHCPIKSRKGDVLHMHYTGKLE--DGTE--FDSSLPQN-------QPFVFSLGTG-QVIKGWDQGLLGMCEGEKRKLVIPSELGYGERGA-PPK-IPGGATLVFEVELLKIE
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FKBP22 VKIEVLQKP-FICHRKTKGGDLMLVHYEGYLEK-DGSL--FHSTHKHNN-----GQPIWFTLGIL-EALKGWDQGLKGMCVGEKRKLIIPPALGYGKEGK--GK-IPPESTLIFNIDLLEIR
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FKBP60_1 LQIERRFVP-DECPRTVRSGDFVRYHYVGTFP--DGQK--FDSSYDRD-------STFNVFVGKG-QLITGMDQALVGMCVNERRFVKIPPKLAYGNEGV-SGV-IPPNSVLHFDVLLMDIW
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FKBP60_2 VQIHTYFKP-PSCPRTIQVSDFVRYHYNGTFL--DGTL--FDSSHNRM-------KTYDTYVGIG-WLIPGMDKGLLGMCVGEKRIITIPPFLAYGEDGD--GKDIPGQASLVFDVALLDLH
FKBP65_2 VQVSTLLRP-PHCPRMVQDGDFVRYHYNGTLL--DGTS--FDTSYSKG-------GTYDTYVGSG-WLIKGMDQGLLGMCPGERRKIIIPPFLAYGEKGY--GTVIPPQASLVFHVLLIDVH
FKBP60_3 ISIENKVVP-ENCERISQSGDFLRYHYNGTLL--DGTL--FDSSYSRN-------RTFDTYIGQG-YVIPGMDEGLLGVCIGEKRRIVVPPHLGYGEEGR--GN-IPGSAVLVFDIHVIDFH
FKBP65_3 VQLETLELP-PGCVRRAGAGDFMRYHYNGSLM--DGTL--FDSSYSRN-------HTYNTYIGQG-YIIPGMDQGLQGACMGERRRITIPPHLAYGENGT--GDKIPGSAVLIFNVHVIDFH
FKBP60_4 ISITSHYKP-PDCSVLSKKGDYLKYHYNASLL--DGTL--LDSTWNLG-------KTYNIVLGSG-QVVLGMDMGLREMCVGEKRTVIIPPHLGYGEAGVD-GE-VPGSAVLVFDIELLELV
FKBP65_4 VEIRTLSRPSETCNETTKLGDFVRYHYNCSLL--DGTQ--LFTSHDYG-------APQEATLGAN-KVIEGLDTGLQGMCVGERRQLIVPPHLAHGESGA-RG--VPGSAVLLFEVELVSRE
C
N
TPR
ER

Figure 3:


H.sapiens MTLRPSLLPLH--LLLLLLLSAAVCRAEAGLETESPVRTLQVETLVEPPEPCAEPAAFGD 58
B.taurus MTLRPSLLPLRLLLLLLLLLRGAVCQAEAGSXTESPVRTLQVETLVEPPEPCAEPATFGD 60
R.norvegicus MTLRPLLLPLR--LLLLLLISGAVCRAEAEVETESPVRTLQVETLVQPPESCTESAAFGD 58
M.musculus MTLSPLLLPLQ--LLLLLLFSGAVCRAEAGPETESPVRTLQVETLVQPPESCTESAAIGD 58
X.laevis MLWG---------FVLLLLLSPATLRAETPEEDSENVTELVIET-VEKPDSCTETAVMGD 50
D.rerio MATRTG-------IVLIFLAVFAFVAAEDGESKESVIEQLVVETLVMP-ESCTITSEMGD 52
H.sapiens TLHIHYTGSLVDGRIIDTSLTRDPLVIELGQKQVIPGLEQSLLDMCVGEKRRAIIPSHLA 118
B.taurus TLHIHYSGSLVDGRIFDTSLTRDPLVIELGQKQVIPGLEQSLLDMCVGEKRRVIIPSHLA 120
R.norvegicus TLHIHYTGSLADGRIIDTSLTRDPLVIELGQKQVIPGLEQSLLDMCVGEKRRAVIPSHLA 118
M.musculus TLHIHYTGSMVDGRIIDTSLTRDPLVIELGQKQVIPGLEQSLLDMCVEEKRRAVIPSHLA 118
X.laevis TIHLHYTGRLEDGRIIDSSLSRDPLVVELGKKQVIPGLETSLVGMCVGEKRKVVIPPHLA 110
D.rerio TLQIHYTGRLMDGKVIDTSLSREPLVVELGKRSVITGLEQALVGVCEGQKIKAMIPAHLA 112
H.sapiens YGKRGFPPSVPADAVVQYDVELIALIRANYWLKLVKGILPLVGMAMVPALLGLIGYHLYR 178
B.taurus YGKRGFPPSIPADAELHFDVELIALIRANYWQKLVKGILPLVGMAMVPALLGLIGYHLYR 180
R.norvegicus YGKRGYPPSIPADAVVQYDVELIALIRANYWQKLLKGILPLVGMAMVPALLGLIGYHLYK 178
M.musculus YGKRGYPPSIPADAVVQYDVELIALIRANYWQKLLKSILPLVGIAMVPALLGLIGYHLYR 178
X.laevis YGKKGYPPSIPGDAVLQFETEVMALFKPTPWQTIVNDVFPLL------HWFGTHAFGAYW 164
D.rerio YGKRGYPPTIPGDSTLEFEVEVISLSQQTPWQKLINDILPLLCLALVPTLLGLVGLYLYN 172
H.sapiens KANRPKVSKKKLKEEKRNKSKKK 201
B.taurus KASSPKISKKKLKEEKRNKSKKK 203
R.norvegicus KASTPRVSKKKLKEEKRNKSKKK 201
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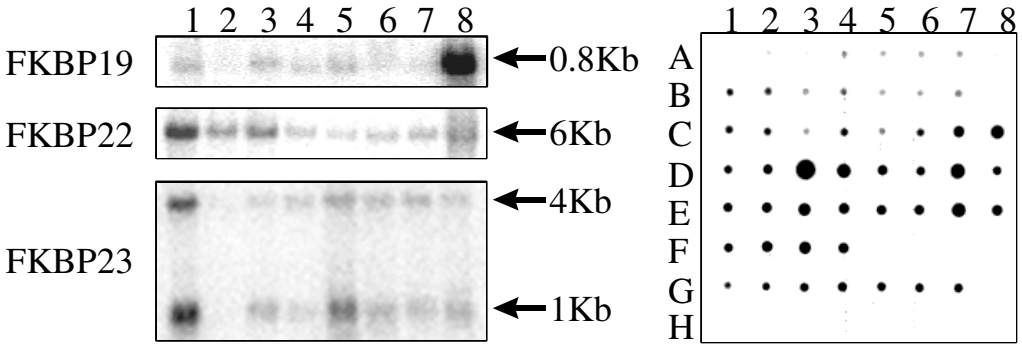
Figure 4: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FKBP22
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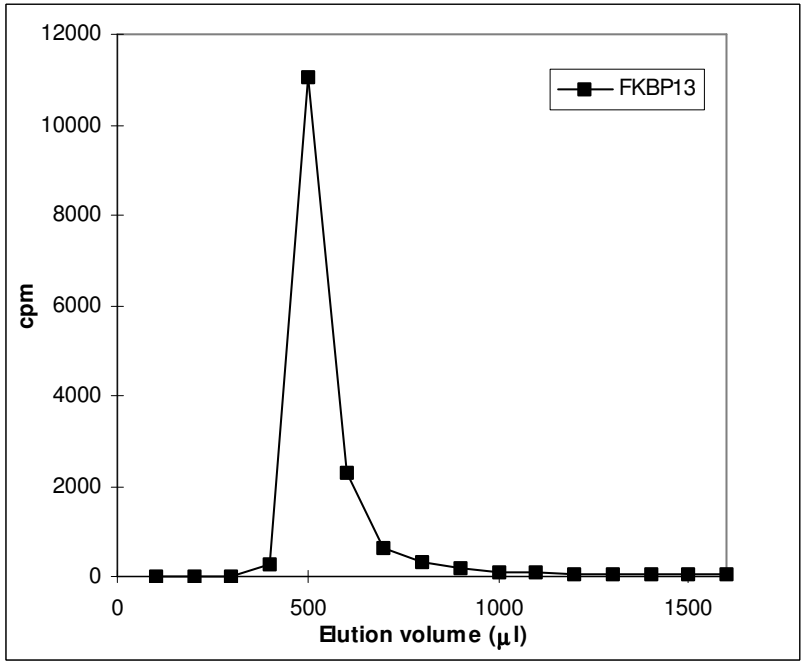
B
C
D
E
F
G
H

Figure 5: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FKBP13
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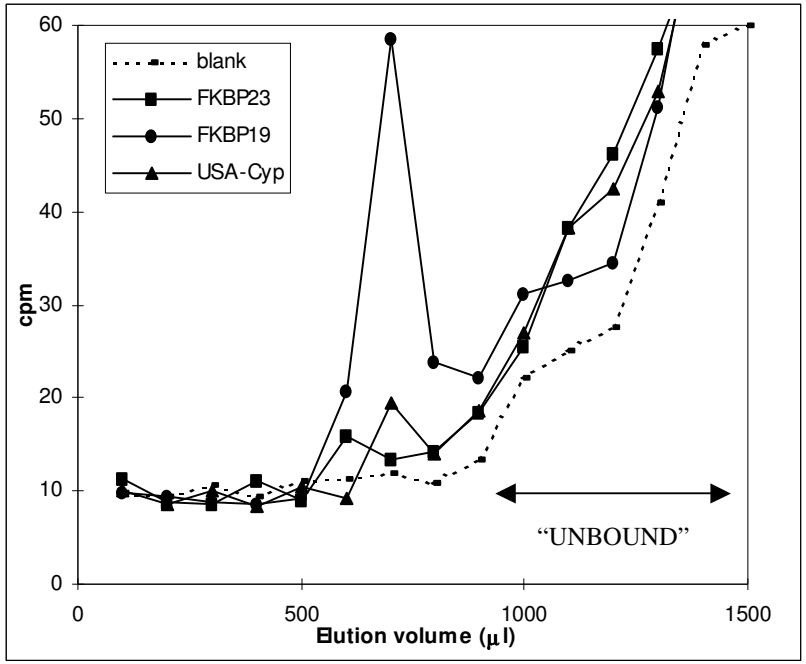
Elution volume (µl)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20
10
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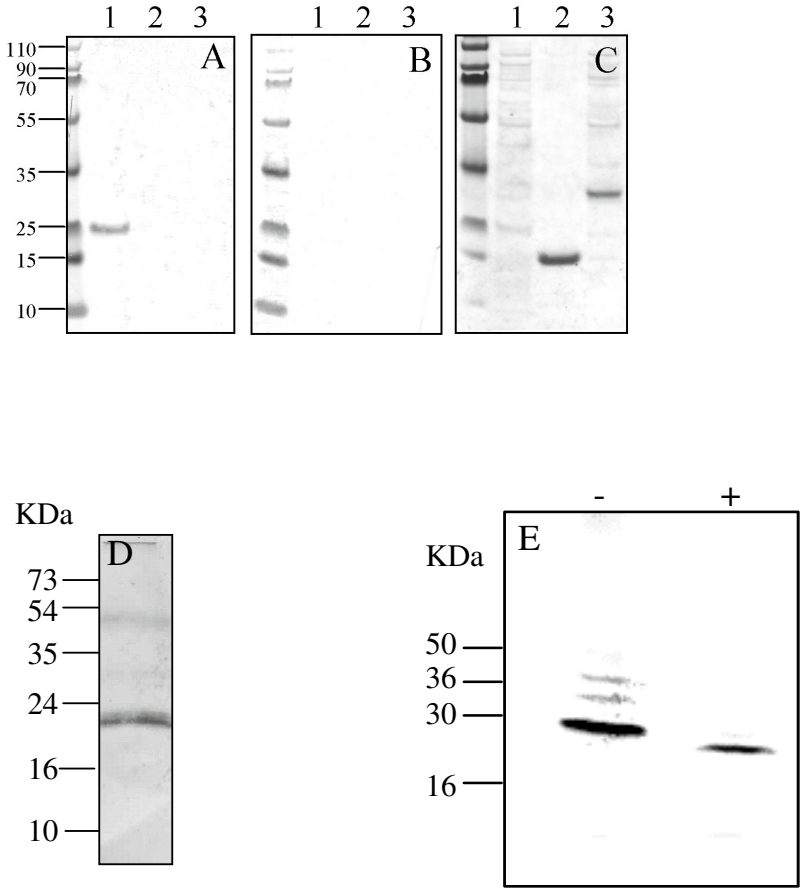
Elution volume (µl)

Figure 6: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16
10
D
-
+
KDa
50
36
30
16
E

Figure 7: